American Fern Journal 98(4):194–198 (2008)

Red Light Inhibition of Spore Germination in *Lycopodium clavatum*

Dean P. Whittier
Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235-1634

Abstract.—Most studies of spore germination in seedless vascular plants have involved species that develop surficial, photosynthetic gametophytes following spore germination. However, several species, including *Lycopodium clavatum*, give rise to subterranean, nonphotosynthetic, mycorrhizal gametophytes and their spores germinate in the dark. Red light, like white light, inhibits the germination of these spores. Germination occurs after exposure to far-red light. The effects of far-red light are reversed by red light and those of red light are reversed by far-red light confirming the involvement of phytochrome. The active form of phytochrome, Pfr, inhibits germination *in L. clavatum*. It appears that this is a general phenomenon in seedless vascular plants with subterranean, mycorrhizal gametophytes because it is now known to occur in two species, *L. clavatum* and *Ophioglossum crotalophoroides*, from unrelated families. The photoinhibition of germination by white or red light insures that these spores germinate underground in nature providing improved chances of spores obtaining adequate soil moisture and mycorrhizal colonization of young gametophytes that are essential for continued development.

Key Words.—*Lycopodium*, spore germination, red light, far-red light, phytochrome

The spores of seedless vascular plants with surficial, photosynthetic gametophytes typically germinate in light (Raghavan, 1989), while those with subterranean, nonphotosynthetic, mycorrhizal gametophytes germinate in the dark (Whittier, 2005, 2006). The germination of spores from ferns of the first group, with one exception (Cooke *et al.*, 1993), is stimulated by red light (Cooke *et al.*, 1987; Raghavan, 1989). The effect of red light on spore germination in a species with underground, mycorrhizal gametophytes has been tested on only one species, *Ophioglossum crotalophoroides* Walter (Whittier, 2006). The germination of these spores, which normally germinate in the dark, is inhibited by red light.

This study was initiated to determine if the inhibition of spore germination by red light occurs in another seedless vascular plant with subterranean, nonphotosynthetic, mycorrhizal gametophytes. In an effort to broaden the study, spores from a family other than the Ophioglossaceae were selected. Spores of *Lycopodium clavatum* L., a species with mycorrhizal gametophytes (Bruchmann, 1910), were chosen because with time they undergo high percentages of germination in culture (Whittier, 1998).

Materials and Methods

Spores of *Lycopodium clavatum* were obtained from plants on Mt. Unaka in Unicoi County, Tennessee. Vouchers are on deposit at the Vanderbilt University Herbarium (VDB). To reduce the incidence of contamination, the

TABLE 1. The effect of daily 30 min exposures to red, far-red, and white light on spore germination in *Lycopodium clavatum*.

Treatment	Spore germination		Percent spore germination
	Yes	No	
Dark	1063	14	98.7
Far-red	1082	47	95.8
Red/far-red	1275	41	96.8
Far-red/red	94	1382	6.4
Red	65	1058	5.9
White	24	1078	2.2

This experiment lasted 110 days and all light treatments had an irradiance of 1.0 mW/cm^2. The percentages of germination for the dark, far-red, and red/far-red light treatments are significantly different from the percentages for the white, red, and far-red/red treatments based on the G test of independence at $p < 0.00001$. Percentages within the white, red, and far-red/red light group and within the dark, far-red, and red/far-red light group are different ($p < 0.01$) but they are not considered biologically significant.

spores were wetted and stored in water for 24 hr before surface sterilization. They were then surface sterilized with 20% Clorox (1.1% sodium hypochlorite) for 2 min by the method of Whittier (1964). Under sterile conditions, the spores were rinsed with water, collected on filter paper, suspended in water and sown on 12 ml of nutrient medium in culture tubes (20 mm x 125 mm) with screw caps that were tightened to reduce moisture loss.

The nutrient medium contained 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, 70 mg K_2HPO_4, and 150 mg NH_4NO_3 per liter. The medium was completed with 2 g of glucose, 0.4 ml of a minor element solution (Whittier and Steeves, 1960) and 4 ml of a FeEDTA solution (Sheat *et al.*, 1959). The medium was solidified with 1.0% agar and was at pH 5.7 before autoclaving. The spores and young gametophytes were cultured at 23±1°C.

Spores were considered germinated once the spore coat ruptured and the proximal cell (near the triradiate ridge) bulged out. The presence of rhizoids cannot be used as an indicator of germination because they form later on multicellular gametophytes. In each case, 1000 or more spores were examined to determine the percentages of germination. These data (Table 1) were analyzed using the G test of independence (Sokal and Rohlf, 1995).

The three experiments carried out in this study had the following light treatments. Red light was obtained with monochromatic red filters (No. 650, Carolina Biological Supply Co.) as in Spiess and Krouk (1977) and white fluorescent lamps. The irradiance used was 1.0 mW/cm^2. Far-red light was obtained with a far-red transmitting plexiglass filter (FRF700, Westlake Plastics Co.) as in Pratt and Cundiff (1975) and incandescent lamps. This light was passed through a Pyrex water cell (10 cm in depth) to reduce infrared radiation and to supply an irradiance of 1.0 mW/cm^2. White light from fluorescent lamps had an irradiance of 1.0 mW/cm^2. The irradiances were measured with a YSI Model 65 Radiometer (Yellow Springs Instruments). Except as otherwise noted, 30 min of red light, 30 min of far-red light, 30 min

of white light were given daily for the red, far-red, and white light treatments. The red/far-red or far-red/red treatments involved a total light exposure of 1 hr/day. In addition to the 30 min white light treatment described above, a 12 hr per day fluorescent white light control (0.3 mW/cm^2) was included with each of the three experiments.

Results

Two preliminary experiments with short durations were conducted to determine if it was feasible to demonstrate a red light effect on spore germination in *L. clavatum*. Red light, white light and a far-red/red treatment inhibited germination in these experiments. Spores in the dark, far-red light and a red/far-red treatment germinated. Because the germination percentages were low with the dark and far-red treatments, another third and final experiment was carried out with a duration of 110 days.

The results of this third experiment are given in Table 1. As found in the previous experiments red light and far-red light had different effects on spore germination in *L. clavatum*. Red light, like white light, inhibited germination and large percentages of germination occurred with far-red light and darkness. When given in sequence red light reversed the effect of far-red light and far-red light reversed the effect of red light. Far-red illumination resulted in germination at essentially the same percentage as spores in the dark. In addition to the results given in Table 1, it was found that spores exposed to a 12 hr/day white light control (0.3 mW/cm^2) failed to germinate. Except for differences in the magnitudes of germination the results were the same for all three experiments.

Discussion

As with *Ophioglossum crotalophoroides* (Whittier, 2006), the photoreversible germination response of *Lycopodium clavatum* spores to red and far-red light indicates the involvement of phytochrome (Toole, 1973). The active form of phytochrome, Pfr, induced by red light inhibits spore germination in both species. These results show that phytochrome is an important component in the germination of spores from *L. clavatum*, as it is with those of *Ophioglossum*.

The 30 min red or white light treatments greatly reduced spore germination in *L. clavatum*, but did not eliminate it as the 20 min exposures did for *Ophioglossum* (Whittier, 2006). Exposing the spores of *L. clavatum* to a 12 hr photoperiod of white light prevented their germination. This raises the possibility that an exposure to red light longer than 30 min is necessary to eliminate spore germination in *L. clavatum*.

The effects of red and far-red light on the spores of *L. clavatum* are essentially the same as those on the spores of *O. crotalophoroides* and opposite those on fern spores from species with photosynthetic gametophytes. Germination is promoted by Pfr, which forms after the exposure to red light in species with

photosynthetic gametophytes (Cooke *et al.*, 1987; Raghavan, 1989), but it is inhibited in the species tested with subterranean, nonphotosynthetic, mycorrhizal gametophytes (Whittier, 2006). Spore germination is not promoted by far-red light in fern species with photosynthetic gametophytes but it occurs in these two species with subterranean, mycorrhizal gametophytes.

The photoinhibition of seed germination insures germination underground rather than at the soil surface which would improve the possibility of sufficient moisture for seedling growth (Salisbury and Ross, 1991). Photoinhibition of *Lycopodium* and *Ophioglossum* spore germination also would insure subterranean germination with improved chances for adequate soil moisture and for enhancing the possibility that young gametophytes will be in the proximity of mycorrhizal fungi. A close association between germinating spores and mycorrhizal fungi is important because young gametophytes of *Lycopodium* and *Ophioglossum* stop growing after a few cells unless colonized by mycorrhizal fungi (Bruchmann, 1910; Campbell, 1911).

White light inhibits spore germination in seedless vascular plant species with subterranean, nonphotosynthetic, mycorrhizal gametophytes (Whittier, 1981, 1998, 2005; Whittier and Braggins, 1994). The photoinhibition of spore germination in *O. crotalophoroides* (Whittier, 2006) and *L. clavatum* is now known to involve Pfr. Because the inhibition of germination by Pfr occurs in two unrelated seedless vascular plant species with subterranean, mycorrhizal gametophytes, it appears that it may be a general phenomenon in this plant group.

Acknowledgments

I thank William Eickmeier and Lee Pratt for comments on this project and Manuel Leal for checking the transmission spectra of the light filters. I am indebted to David McCauley for assistance with the statistics.

Literature Cited

Bruchmann, H. 1910. Die Keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L., *L. annotinum* L., und *L. selago* L. Flora 101:220–267.

Campbell, D. H. 1911. The eusporangiate. Carnegie Inst. Publ. Wash. No. 140.

Cooke, T. J., R. H. Racusen, L. G. Hickok and T. R. Warne. 1987. The photocontrol of spore germination in the fern *Ceratopteris richardii*. Plant Cell Physiol. 28:753–759.

Cooke, T. J., L. G. Hickok, W. J. Vanderwoude, J. A. Banks and R. J. Scott. 1993. Photobiological characterization of a spore germination mutant *dkg*1 with reversed photoregulation in the fern *Ceratopteris richardii*. Photochem. Photobiol. 57:1032–1041.

Pratt, L. H. and S. C. Cundiff. 1975. Spectral characterization of high-molecular-weight phytochrome. Photochem. Photobiol. 21:91–97.

Raghavan, V. 1989. *Developmental biology of fern gametophytes*. Cambridge University Press, Cambridge, U.K.

Salisbury, F. B. and C. W. Ross. 1992. *Plant physiology*, 4th Ed., Wadsworth Publishing Co., Belmont, Calif.

Sheat, D. E. G., B. H. Fletcher and H. E. Street. 1959. Studies on the growth of excised roots. VII. The growth of excised tomato roots supplied with various inorganic sources of nitrogen. New Phytol. 58:128–154.

SOKAL, R. R. and F. J. ROHLF. 1995. *Biometry*. 3rd Ed. W.H. Freeman and Co., New York.

SPIESS, L. D. and M. G. KROUK. 1977. Photocontrol of germination of spores of the fern *Polypodium aureum*. Bot Gaz. (Crawfordsville) 138:428–433.

TOOLE, V. K. 1973. The effects of light, temperature and their interactions on the germination of seeds. Seed Sci. Technol. 1:339–396.

WHITTIER, D. P. 1964. The effect of sucrose on apogamy in *Cyrtomium falcatum*. Amer. Fern J. 54:20–25.

WHITTIER, D. P. 1981. Spore germination and young gametophyte development of *Botrychium* and *Ophioglossum* in axenic culture. Amer. Fern J. 71:13–19.

WHITTIER, D. P. 1998. Germination of spores of the Lycopodiaceae in axenic culture. Amer. Fern J. 88:106–113.

WHITTIER, D. P., J.-C. PINTAUD and J. E. BRAGGINS. 2005. The gametophyte of *Lycopodium deuterodensum* – Type II or I. Amer. Fern J. 95:22–29.

WHITTIER, D. P. 2006. Red light inhibition of spore germination in *Ophioglossum crotalophoroides*. Canad. J. Bot. 84:1156–1158.

WHITTIER, D. P. and J. E. BRAGGINS. 1994. Spore germination in the Psilotaceae. Canad. J. Bot. 72:688–692.

WHITTIER, D. P. and T. A. STEEVES. 1960. The induction of apogamy in the bracken fern. Canad. J. Bot. 38:925–930.